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Original
Process Analytical Technology for monitoring pharmaceuticals freeze drying - A Comprehensive Review / Fissore, D.; Pisano, R.; Barresi, A. A.. - In: DRYING TECHNOLOGY. - ISSN 0737-3937. - STAMPA. - 36:15(2018), pp. 1839-1865. [10.1080/07373937.2018.1440590]

Availability:
This version is available at: 11583/2704855 since: 2020-02-02T19:06:06Z

Publisher:
Taylor & Francis

Published
DOI:10.1080/07373937.2018.1440590

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12 October 2024

Drying Technology [ISSN: 0737-3937], 36 (15), 1839-1865. DOI: 10.1080/07373937.2018.1440590

Process Analytical Technology for Monitoring Pharmaceuticals Freeze Drying - A Comprehensive Review

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Abstract

The goal of this paper is to review the available Process Analytical Technology tools for monitoring the batch freeze-drying process for pharmaceuticals. These systems aim evaluating in-line product temperature, the sublimation flow rate, and the values of some model parameters in such a way that it can be used for in-line or off-line process optimization. A detailed survey of the systems proposed in the literature is presented, grouping them on the basis of the monitored variable, namely product temperature, the heat flux to the product, the sublimation flux, and, finally, other variables. The advantages and the drawbacks of the techniques are critically assessed, taking into account the possibility of using them not only at lab-scale, but also at production scale, beside, obviously, the possibility of getting reliable measurements of the desired variables.

Keyword

Freeze-drying, Process Analytical Technology, thermocouple, soft-sensor, pressure rise test, product quality.

Introduction

Freeze-drying is a process where a product is dried through the sublimation of the frozen liquid. It is a key step in pharmaceuticals manufacturing: in fact, liquid removal is required due to the higher stability of drugs at solid state than in liquid solution, and freeze-drying allows preserving most of the critical quality attributes of the product (if the process is properly carried out, as it will be discussed in the following). Moreover, at the end of a freeze-drying process a highly porous cake of dried product can be obtained, and it can be easily reconstituted.[1]-[4]

These issues motivate the exponential increase of the number of products requiring freeze-drying in the manufacturing process: in 2016, 50% of the novel drugs approved by the Food and Drug Administration (in USA) were biopharmaceuticals[5], and about 50% of the approved biopharmaceutical drugs on the list of the FDA and of the European Medicines Agency are freeze-dried.[6]

A typical freeze-drying process encompass three stages:

1) Freezing: product temperature is decreased in such a way that the solvent freezes. Although the use of organic solvents, e.g. tert-butyl alcohol in combination of water, was reported in the literature, and can be found also in the manufacturing of commercial products (e.g. Aprostatil, Amoxicillin sodium and Imexon[7]-[9]), in most cases water is the solvent that has to be removed. Only a fraction of the water in the system, the “free water”, freezes, and the remaining, called “bound water”, remains bound, at liquid state, to the product molecules.
2) Primary drying: in this stage the sublimation of the frozen solvent takes place. The pressure in the environment surrounding the product is decreased well below the triple point of the solvent, and heat is supplied to the product as the ice sublimation is an endothermic process.

3) Secondary drying: once the ice has been removed from the product, it is necessary to remove also the bound water, aiming to get the target value of residual moisture in the final product. This is accomplished by further supplying heat to the product to accelerate the process of water desorption from the dried product.

These three stages are usually carried out in a batch freeze-dryer: the liquid product is poured into vials, syringes, or in trays (in case of bulk freeze-drying), and these containers are placed onto the shelves of the drying chamber. A technical fluid flows inside these shelves, removing heat from the product during the freezing stage, and heating it during the drying stages. The desired vacuum level is obtained with a vacuum pump and a condenser, where the solvent vapor leaving the product is frosted. Additionally, a tight regulation of chamber pressure can be obtained introducing a controlled flow rate of inert gas in the chamber (controlled leakage). Drying chamber and condenser can be connected through a duct, where a valve is usually present, and used to isolate the drying chamber from the condenser for monitoring purposes, as it will be discussed in the following.[1]-[4]

The target of the process is to get the desired value of residual moisture in the final product. Besides, the rate of solvent removal should be maximized, aiming to maximize plant productivity, and reducing the cost of the product. It is thus necessary to monitor, during the drying stages, the residual moisture in the product, to identify (i) the ending point of the primary drying stage, and (ii) the ending point of the freeze-drying process. It is in fact necessary to understand when the ice sublimation is completed as in the secondary drying stage product temperature is further increased and, thus, the presence of residual ice is unwanted.

Two constraints must be fulfilled during the operation:

1) The temperature of the product has to remain below a limit value that is a characteristic of the product being processed. In case of crystallizing products, the

limit value is the eutectic temperature, to avoid the formation of a liquid phase. Beside product temperature, it has also been proposed to consider the electrical conductivity of the solid, to have a higher sensitivity and prevent the melting phenomenon.[10] In case of amorphous products, the limit value is the collapse temperature, to prevent the collapse of the dried cake, that may cause a slowing down in the process (due to the blockage of the pores of the cake where the vapor has to flow), a higher reconstitution time, a higher water content in the final product, and even the loss of activity of the active pharmaceutical ingredient.[11]-[17] Instead of the collapse temperature, the glass transition temperature, a couple of degrees lower, is usually considered as the limit value in a precautionary way.

2) The rate of vapor production in the drying chamber has to be compatible with the condenser capacity, to avoid vapor accumulation, and subsequent pressure increase, in the drying chamber. Besides, choking flow has to be avoided in the duct as, also in this case, pressure increase in the chamber would occur.[18]-[21]

Summarizing, after having characterized both the product, identifying the limit temperature, and the freeze-dryer, evaluating the duct and condenser capacity, it is necessary to monitor the residual amount of ice in the product, its temperature, and the sublimation flow rate, through suitable Process Analytical Technologies (PAT), thus evaluating in-line if the desired quality is obtained in the final product. This approach is totally different from the traditional quality-by-testing, and it was strongly encouraged by the "Guidance for Industry PAT - A Framework for Innovative Pharmaceutical Development, Manufacturing, and Quality Assurance" issued by FDA in September 2004 with the goal of producing effective, safe, and affordable medicines.[22]

The monitoring system should be able to estimate in-line also the values of some parameters of a mathematical model describing the dynamics of the process, so that the model

can then be used for optimizing the process in-line, using a model-based control system[23]-[25], or off-line, through the calculation of the design space[26]-[28]. The first parameter usually desired is the heat transfer coefficient K_v, used to express the dependence of the heat flux to the product (J_q) on the driving force given by the difference between the temperature of the heating shelf (T_{shelf}) and that of the product at the bottom of the container (T_B):

$$J_q = K_v \left(T_{shelf} - T_B \right) \tag{1}$$

The second parameter is R_p, the resistance of the dried cake to vapor flux, used to express the dependence of the solvent flux (J_w), during the primary drying stage, on the driving force given by the difference between solvent partial pressure at the interface of sublimation ($p_{w,i}$) and in the drying chamber ($p_{w,c}$):

$$J_w = \frac{1}{R_p} \left(p_{w,i} - p_{w,c} \right) \tag{2}$$

Previous equations were used in a simplified model of the process[29], extensively used for in-line and off-line process optimization[23],[30]. With respect to the secondary drying stage, the desired parameter is the kinetic constant (k_d) used to model the dependence of the desorption rate on the residual solvent concentration through one of the following equations[31]-[33]:

$$r_d = a k_d C_s \tag{3}$$

$$r_d = a k_d \left(C_s - C_{s,eq} \right) \tag{4}$$

Much more detailed models were also proposed to describe product dynamics in the process, for both the primary and the secondary drying stages (see for example the distributed sublimation front based model[34], and the multilayer water state in the solid matrix[35]), but they are characterized by a higher number of parameters that have to be estimated, and the accuracy of these estimates can jeopardize the higher accuracy of the model.

An additional issue that has to be faced by the monitoring system is the fact that product evolution is not the same among the various vials of the batch. This is a consequence

of the different mechanisms of heat transfer to the product (e.g. vials of the first rows are heated also by radiation from chamber walls, and not only through the shelf)[36]-[38], of the gradients of pressure in the chamber[39], and of the non-uniform nucleation rate[40]. Therefore, product temperature, the residual amount of ice, and the values of the model coefficients can vary according to the position of the vial in the batch. This make process monitoring much more difficult, because it is not sufficient to monitor a single vial, but a selection representative of the whole batch must be considered. The other consequence is that the end of primary drying does not occur at the same time in all the vials, but a distribution curve exists, and the time corresponding to the complete drying of the largest part of the batch is usually identified, although this depends on the type of sensor used. As the heterogeneity of the batch depends also on batch scale, transfer of the monitoring procedures, and of the results obtained in the process development step, at larger scales must be handled with great caution.[41]

Various analytical techniques were proposed to monitor the freeze-drying process. Some of these have been used for in-situ characterization of product in single vials (or even capillary) during freeze-drying, but require a special lyophilization equipment, connected with the analytical instrument. Low temperature X-ray powder diffractometry[42],[43], low-resolution pulse Nuclear Magnetic Resonance[44], FTIR spectroscopy[45], optical coherence tomography based freeze-drying microscopy[46],[47] and visual microscopic observation[48] are some examples. This paper aims reviewing the current PAT proposed to monitor the freeze-drying process, which can be employed in a conventional freeze-dryer with no or minor modifications. Other reviews were published in the last years on this topic: Barresi et al.[49] discussed monitoring of primary drying, Johnson et al.[50] assessed sensitivity and reliability of sensors for primary drying and reviewed methods for characterizing the freeze-dried cake, Wiggenhorn et al.[51] presented a few novel methods to determine the status of lyophilized

formulations, Patel and Pikal[52] presented an overview of the challenges associated with each step of the freeze-drying process, while Patel et al.[53] focused on determination of end point of primary drying in freeze-drying process control. All the three steps of freeze-drying process have been analyzed by Barresi and Fissore[54] with attention to the assurance of in-line quality by monitoring and control, presenting possible solutions based either on the average batch monitoring, or on single vial monitoring. It was pointed out that measuring devices should be able to supply the full state of the system, in order to be applicable in a predictive control system. Recommended best practices for process monitoring instrumentation have been very recently summarized by the LyoHub consortium[55], including also a brief survey of the technology currently under development and that may become worthy of analysis in the next future. It is also important to remind that often it may be recommendable to rely on complementary technique when monitoring critical material like high-concentrated amorphous material.[55],[56]

The various techniques proposed can be catalogued depending on the size of the sample, as they can be used to monitor single vials, a group of vials or the whole batch (see e.g. Refs. [49] and [54]); in this work they have been grouped on the basis of the monitored variables: at first, the system based on product temperature measurement are presented and discussed, including also those based on the measurement of the heat flux. Then, monitoring systems based on the pressure measurement during the pressure rise test are presented and, finally, those based on the measurement of the sublimation flux. Afterwards, the systems monitoring a specific physical property related to temperature or moisture, and those available to identify the ending point of the primary drying stage, are reviewed, with those proposed for the secondary drying stage. In conclusion, the application of these systems to industrial scale units is discussed.

Monitoring systems based on product temperature or heat flux measurement

Product temperature can be easily monitored through a temperature sensing device: thermocouples or resistance thermal detectors (RTD) inserted in the vial, in contact with the product, are the most widely used, but other sensors have been recently proposed. Thermocouples are generally used in lab-scale freeze-dryers[57], while RTD are most frequently used in production freeze-dryers as they are more robust and can be sterilized[58],[3], although their size may affect the accuracy of the measurement[59]. In addition, in case of thermocouples if thin wires are chosen the sensing tip can very small, and this allows a punctual temperature measurement and an easier and more accurate positioning in the batch; but, of course, the measure becomes very sensitive to the correct location, and even a very small displacement can affect the result. On the other hand, a larger sensing device measures a larger portion of the product, giving an average value, less accurate but more robust. In general, a good compromise must be found, considering also the mechanical robustness of the device and of wires.[55]

Optical fiber sensors (OFS) are widely used in several fields, and have been recently proposed also for monitoring the product temperature during a freeze-drying process.[60] The sensing element is a fiber Bragg grating with periodical variation of refractive index: as the refractive indices of FBGs are temperature dependent, the measured reflection can be related to local temperature. Optical fiber sensors can be used for invasive monitoring (immersing the fiber in the product), and, in this case, it is also possible, with an helix arrangement, to measure the 3D temperature profile, or non-invasively, fusing the fiber in the vial bottom. They are steam-sterilizable, and proved to be more sensitive than thermocouples, with faster response time and better resolution.

The Temperature Remote Interrogation System (TEMPRIS) sensors are another type of device used for temperature monitoring[61]: they are passive transponders, receiving energy

from an electromagnetic field, thus eliminating the necessity of wire connections, even if the freeze-dryer must be properly equipped in order the sensors may be correctly irradiated in all the positions of interest. The main disadvantage of TEMPRIS is the very large size of the device, which strongly affects not only the freezing step, but also the drying, as the height of the filled volume significantly increases if the sensor is completely immersed, so that the measuring tip is positioned on the bottom of the vial.

When using one of the temperature sensors just described to monitor the freeze-drying process, there are several important issues that have to be taken into account:

1) The presence of the sensor in the vial, immersed in the liquid solution at the beginning of the process, may affect ice nucleation and, thus, the size of the ice crystals. This may, in turn, affect the dynamics of the product in the monitored vial (a 10% increase of the drying rate was reported[55]) as the features of the dried cake, mainly the size of the holes available for vapor flow from the interface of sublimation, corresponds to the size of the ice crystals. As a consequence, the temperature in the monitored vial can be different from that of the other vials of the batch. Anyway, at least at lab-scale, in non-GMP (Good Manufacturing Practice) conditions, this effect is negligible for small volume sensors, as it was pointed out by Bosca et al.[62]. As discussed, the large volume of the TEMPRIS can cause significant modifications of the total volume, if completely immersed; for this reason, the performance of the sensor was investigated when only the tip is immersed or even when it is above the product: it was shown that the absolute temperature measured changes, but it has been claimed that it is possible to use it to detect the end of drying in all cases. Anyway, it has been shown that the presence of the sensor in the vial, even not in contact, may cause uncontrolled freezing.[63]
2) The insertion of the probe in the product, and the handling and positioning of the sensors itself, can compromise the sterility of the system. Besides, the use of wired

probes it is not compatible with the automatic loading/unloading systems used at production scale.[64],[61]

3) When using a temperature probe it is not possible to monitor product temperature till the end of the primary drying stage. In fact, at a certain point during the primary drying stage, and before the ending point, the temperature value detected through the thermocouple starts increasing unexpectedly, rising rapidly to the temperature of the heating shelf. This would be expected in case the ice sublimation is completed in the monitored vial, as all the heat provided to the product would be used to increase its temperature, but, as it was evidenced by Chen et al.[65] and Bosca et al.[62], when this temperature increase occurs there is still some ice in the monitored vial, and the ice sublimation is not completed in the batch. Figure 1 shows an example of this occurrence, where the temperature measurement is compared to the ratio of the signals obtained through a capacitance (Baratron type) and a thermoconductive (Pirani) gauge: when this ratio starts decreasing it means that ice sublimation is approaching the ending point, as the composition of the gas in the drying chamber moves from about 100% water vapor to about 100% air (or nitrogen, in case controlled leakage is used for pressure control).[64] Figure 1 shows that the sudden temperature rise is detected well before the moment when the pressure ratio starts decreasing and, thus, well before the ending point of the primary drying stage. Probably, the sudden change of temperature is due to the loss of contact between the product and the probe tip, or to the fact that the moving sublimation interface advances past the probe tip. In any case, when the temperature change occurs, the following measurements are no longer representative of the frozen product temperature.

4) By placing sensors in several vials of the batch, e.g. in vials of the first rows and in the center of the batch, it is possible to track batch dynamics accounting for its non-

uniformity[66],[67], as it is shown in Figure 1, graph A. In any case, it is critical the positioning of the sensor inside the vial, because the reading strongly depends on the distance from the bottom, and the measures can be affected by off-center displacement; for this reason, it is recommended to use a device for positioning and maintaining the probe in the right position, and several of them were also patented.[68]-[70]

Some of the previously listed drawback, related to the use of wired and invasive probes, may be solved through some recent technological innovations. As an example, wireless sensors may be used with automatic loading/unloading systems, even if the sterile insertion of an instrumented vial in the loading line requires always a non-trivial procedure. The TEMPRIS sensor has been already described, but its use has several drawbacks and the size of the probe is a problem. A different device was proposed by Corbellini et al.[71],[72] and by Bosca et al.[66]: it is a battery powered device, equipped with thermocouples and an embedded radio operating at 2.4 GHz, assuring the radio communication over 10-20 m range from the receiver.

As concerns non-invasive sensors, plasma sputtering was proposed to embed thin film sub-micrometric thermocouples in the glass wall, without affecting the inner surface of the vial, thus avoiding any interaction with the product[73]-[75]; it is also possible to realize sputtered thermocouple arrays for a more accurate monitoring.[76],[77] A different solution is the use of optical fibers with the fiber Bragg gratings embedded in the shelf[60]: obviously, in this case the correct positioning of the vial above the FBG is crucial for a correct monitoring. The use of an infrared thermocamera, placed outside the freeze-dryer, was also proposed by Hemteborg et al.[78] to monitor product temperature over the top shelf of the freeze-dryer without any interference with the product, and, obviously, without using any special vials.

As it was outlined in the Introduction of this paper, not only product temperature, but also the ending point of the primary drying stage and model parameters K_v are R_p should be

monitored in-line. When using a thermocouple, it is possible to identify the ending point of the primary drying stage looking for the time instant when the temperature detected becomes equal to the temperature of the heating source. Actually, it is not very easy to identify the exact ending point, taking also into account that several temperature measurements can be available in the freeze-dryer, and batch non-uniformity plays an important role. Anyway, the uncertainty in the detection of the ending point during the primary drying stage is not higher than that associated to other devices, e.g. the pressure ratio.[53]

Thompson[79] proposed to use a temperature sensor to detect the end of primary drying, by regularly and drastically reducing the pressure in the chamber: if a decrease of product temperature is measured, it indicates the presence of residual ice in the vial.

As concerns the identification of model parameters a simple approach can be used. The heat transfer coefficient K_v may be calculated using the following equation, obtained from the heat balance for the frozen product:

$$K_v = \frac{m\Delta H_s}{A_v \int_0^{t_{drying}} \left(T_{shelf} - T_B\right) dt} \tag{5}$$

where m is the mass of ice in the vial, A_v is the cross-section area of the vial, and t_{drying} is the time required to complete the ice sublimation. As the temperature measurement is not available up to the ending point of the primary drying stage, it is possible to make the hypothesis that the slope of the temperature profile does not change if the temperature of the heating source and the pressure in the chamber are not modified. Figure 2 shows an example of the values of K_v calculated by this way, using 4 different temperature measurements, and the value obtained through the classic gravimetric test[21], evidencing the adequacy of the method. With respect to R_p, once K_v is known, the parameter can be easily calculated by this way:

i. Using the measured values of T_{shelf} and T_B and the calculated value of K_v, the heat flux,

at each time instant, is calculated with eq. (1).

ii. The sublimation flux, at each time instant, is calculated from the energy balance at the interface of sublimation:

$$J_w = \frac{J_q}{\Delta H_s} \tag{6}$$

iii. R_p is finally calculated, at each time instant, using eq. (2), as J_w is known, $p_{w,c}$ is assumed to be equal to chamber pressure (being the gas in the chamber almost exclusively composed by water vapor), and calculating $p_{w,i}$ from the temperature at the interface of sublimation, assuming that it is equal to the measured one.

iv. L_{dried}, at each time instant can be then calculated from the following mass balance:

$$\frac{dL_{dried}}{dt} = \frac{1}{\rho_{frozen} - \rho_{dried}} J_w \tag{7}$$

and, finally this allows obtaining R_p as a function of L_{dried}.

Figure 3 shows an example of the results obtained using this method, evidencing that also in this case we get several curves, one for each temperature measurement used, and this allows evaluating a mean value of the parameter, with an uncertainty range.

As an alternative, it is possible to use a soft-sensor. It is an algorithm based on the following system of equations:

$$\begin{cases} \begin{pmatrix} \dfrac{d\hat{T}_i}{dt} \\ \dfrac{d\hat{K}_v}{dt} \end{pmatrix} = \begin{pmatrix} \mathbf{f}\left(\hat{T}_i, \hat{K}_v, T_{shelf}\right) \\ 0 \end{pmatrix} + \mathbf{K}\left(\hat{T}_B - T_B\right) \\ \hat{T}_B = h\left(\hat{T}_i, \hat{K}_v, T_{shelf}\right) \end{cases} \tag{8}$$

that estimates in-line T_i and K_v using the measured value of T_B, and calculates R_p and L_{dried} with an approach similar to that previously described (the detailed equations can be found in Ref. [80]), where $\hat{T}_i$ is the estimate of product temperature at the interface of sublimation, $\hat{K}_v$

is the estimate of the heat transfer coefficient, $\hat{T}_B$ is the estimated product temperature at the bottom of the container, and **K** is the gain of the soft-sensor, calculated using the extended Kalman filter algorithm. By this way there is no need to calculate K_v separately, as this parameter is estimated in-line. An example of the results is shown in Figure 4, evidencing that the algorithm correctly estimates product temperature till the ending point of the primary drying stage, and that the ending point is in good agreement with that estimated using the pressure ratio.

A soft-sensor using the measurement of product temperature and a neural network based mathematical model was proposed by Dragoi et al.[81],[82]: in this case a simple neural network is trained off-line, and it allows a fast in-line estimate of the drying time and of model parameters K_v and R_p.

Not only the temperature, but also the heat flux can be used to monitor the lyophilization process and to detect the end of the primary drying. This technique is closely related to those just described, as it also uses thermocouples and is based on the use of eqs. (1) and (5). In particular, Jennings and Duan[83] proposed to monitor primary drying measuring the total energy required by the process: they used two thermocouples fixed to the bottom of an empty vial and of a vial filled with the product, and they calculated the heat transfer to the filled vial used for the sublimation of ice. The end of primary drying was indicated by a drop of the heat transfer rate.[2] The differential method, named Drying Process Monitoring (DPM), requires the preliminary determination of the heat transfer coefficient in the monitored vial, by means of a calorimetric measurement. This is more sophisticated, and probably more reliable, than the single temperature measurement for primary drying, but it presents all the limitations of the wired thermocouples. It was claimed to be usable also for detecting the end of secondary drying, but, in this case, it is probably not very sensitive.

Devices have been developed to measure directly the heat flux from the shelf to the

vial bottom, thus allowing to monitor the drying process and to detect the end of primary drying (while they are not valuable for the end of secondary drying). They are arrays of paired thermocouples, with thermocouples in a pair mounted close to the top and bottom surfaces of the transducer, that is only a few tenths of millimeter thick; they are oriented in different directions, and allow to account for different contributions, including radiative heat, even if only partially and of the heat flow direction [65],[84]. A critical issue is the positioning of the vial and the thermal contact of its bottom, while the sensor itself allows a non-invasive measurement, and should not alter significantly the operating conditions for the monitored vials. Chen et al.[65] substituted the glass bottom of the monitored vial with a flat copper bottom, to improve thermal contact, but this can bias the reliability of the measurements. Recently, Vollrath et al.[84] have evaluated and improved a similar system, commercially available, that utilizes a thin foil-type thermopile, that is an array of approximately 50-60 differential thermocouples connected in series.[85]

The heat flux sensors can be useful also for monitoring the freezing step, evidencing the onset of nucleation. They do not require a previous evaluation of K_v, the heat transfer coefficient, but this parameter is necessary if also the temperature value is desired. The drying time estimated by these devices was shown to be shorter than that obtained using the comparative pressure measurement method, but the last value is known to be influenced by last vials to dry and by the hydrodynamics in the apparatus. Certainly, also in this case there is a problem of representativeness of the sample: in fact, a single vial, a group of vials (using a sensor of larger area) or even multiple vials in different positions on the shelf can in principle be measured.

Monitoring systems based on measurement of the sublimation flux

This section is focused on all those methods that can give a direct measurement of the

sublimation rate. These methodologies have been divided into two groups, namely (i) those based on the Pressure Rise Test (PRT), that introduces a perturbation into the input so as to determine the parameters of interests, and (ii) all the other methods. Special emphasis is given to the PRT since it received very much attention in the literature.

Methods based on the Pressure Rise Test

The Pressure Rise Test is a class of methods that has been proposed for the monitoring of the dynamics of both primary and secondary drying. PRT has been inspired by the step test procedure which is widely used in control practice for the development of empirical models from process data. The step test procedure is essentially based on the analysis of the process reaction curve, i.e. the output response of the process, resulting from a step change in input. As concerns the PRT method, a step change in input is realized by implementing a sudden change in the position of the on-off valve placed on the duct connecting the drying and condenser chambers. This corresponds to a step change in outflow, i.e. the vapor flow rate evacuated from the drying chamber; more specifically, the drying chamber is isolated from condenser and thus outflow is forced to be zero, thus resulting in an increase in chamber pressure. To provide an example, Figure 5 shows the PRT curve as observed in a laboratory scale freeze-dryer during primary drying of a sucrose-based formulation.

The increase in pressure, as observed right after the shut-off of the on-off valve, is correlated to the vapor flow rate and, more specifically, to the rate of sublimation or desorption depending if PRT is performed during primary or secondary drying respectively. Therefore, the sublimation flux, or of desorption, corresponds to the slope of the step response at the origin:

$$J_w\big|_{t=0} = \frac{VM_w}{N_v A_v R T_{gas}} \frac{dp_{w,c}}{dt}\bigg|_{t=0} \tag{9}$$

where the starting time, i.e. the time at which the step change is implemented, is arbitrarily set

to 0. If PRT is regularly performed during a freeze-drying cycle, the vapor flow rate can be monitored. This information is widely used in freeze-drying practice to detect the completion of ice sublimation as the time at which the slope of the pressure rise curve, or equivalently the rate of sublimation, is below a given value, that is sufficiently small to be negligible or comparable to the leakage in the apparatus.[86] Neumann also observed that the steady-state value of the pressure rise curve is tightly related to the ice vapor pressure and, hence, to the product temperature at the moving interface. The larger the ultimate value of pressure in response to the step change in outflow, the higher the product temperature; the converse is of course also true. However, it must be observed that the increase in pressure produces an increase in the heat transfer efficiency between the equipment and the vials where sublimation is occurring, and a decrease in the driving force for the mass transfer, as given by the difference between the ice vapor pressure and the partial pressure of water inside the drying chamber. The influence of the former contribution on the product dynamics is negligible, provided that the duration of the test is limited to few minutes or less, whereas the decrease in the driving force has an immediate effect on the rate of sublimation. It follows that, if the heat transferred to the product remains unchanged, while that removed by sublimation progressively decreases, the product temperature increases during a PRT. Consequently, product temperature, as obtained from the steady-state pressure value, is much higher than that observable before a test. This increase in product temperature is product specific and tightly related to the resistance to mass transfer R_p since this parameter correlates the vapor flow rate and its driving force, see Eq. (2). Pisano et al.[23] observed that the temperature increase is usually in the range of 1-3 °C for a test lasting 30 s. A further refinement of the regression of product temperature from the PRT was given by Oetjen et al.[87] and Oetjen and Haseley[3]. They proposed an algorithm, named Barometric Temperature Measurement (BTM), that estimates the product temperature from the value of pressure at which the first

derivative pressure rise curve exhibits its maximum. This modification improves the accuracy of the product temperature estimations, which however are still overestimated when compared to experimental data as obtained by thermocouples.

Both BTM and the method proposed by Neumann give a rough estimation of the product temperature at the interface of sublimation, while they do not give any information about the temperature profile within the product being lyophilized and, particularly, that close to the vial bottom where the product exhibits its maximum. This last temperature is of upmost importance to develop successful control strategies for primary drying. In 1997, Milton et al.[88] were the first ones to use a mathematical model, describing the product dynamics, to analyze the pressure rise curve. Their method is commonly referred to as Manometric Temperature Measurement (MTM) and can estimate, besides the product temperature at the interface of sublimation[89], the resistance to vapor flow through the dried layer[90] and the heat transfer coefficient[91]. However, the mathematical model used by MTM is based on some strong hypotheses that might be source of errors[92]:

- The frozen layer is thermally insulated at the top from the dried layer and at the bottom from the vial.
- The model calculations require that the temperature gradient along the frozen layer is known. Since this information is not available, it is usually fixed to 2°C without accounting for the fact that its value varies, e.g., with the thickness of frozen layer and hence as the drying proceeds. This problem was partially solved by Tang et al.[89]-[91] who have correlated the above temperature gradient to processing conditions and other outcomes of the MTM algorithm.
- The heat capacity of the container is completely neglected. This contribution has successively been included by Obert et al.[93], but was found to be irrelevant to the parameters of interest.

Since its first publication in 1997, MTM received great attention from the lyophilization community because it represented the first alternative to thermocouples as a non-invasive tool for the monitoring of the product temperature. Besides, the possibility of estimating heat and mass transfer coefficients, and their use in model-based control tools, allowed this methodology to gain industrial acceptance in recent years.[55]

Given the potentiality of this method, various authors followed it through with the development of more sophisticated models that give a more accurate description of the product dynamics and, hence, of the pressure response of the system to the step change in outflow. Liapis and Sadikoglu[94] proposed the use of a detailed, unsteady-state model[33] for the analysis of the pressure rise curve; this algorithm is known as Dynamic Pressure Rise (DPR). DPR requires the knowledge of many parameters, e.g. diffusivity and permeability of vapor through the dried porous layer and the heat transfer coefficient at the vial bottom, which makes its application difficult in practice. More recently, Chouvenc et al.[95] proposed a simpler algorithm, named Pressure Rise Analysis (PRA), which uses a macroscopic model, based on the heat balance within the frozen product, for the analysis of the pressure response. Beside the product temperature at the interface of sublimation, PRA estimates the heat and mass transfer resistances and the rate of desorption of bounded water. This last parameter is however useless since it is commonly negligible during primary drying. The main drawback of this algorithm is that product temperature is assumed to be constant during a PRT and, as we have discussed above, this assumption is not valid for all those situations wherein pressure increases significantly during a PRT, e.g. full load conditions, aggressive cycles, etc... A further improvement to the analysis of the pressure response was achieved by Velardi et al.[96] through the Dynamic Parameters Estimation (DPE) algorithm which uses a mono-dimensional unsteady-state model of the process. DPE can estimate heat and mass transport properties as well as the temperature profile through the frozen product and its evolution

during a PRT. This last information is essential for the in-line process control, particularly for those cycle that are carried out close to the maximum allowable product temperature[97]. The DPE algorithm can also give a rough estimation of batch heterogeneity[98] and account for side-wall radiation[99]. This last feature is essential for all those cycles wherein radiation from chamber walls substantially contributes to the total heat transfer, e.g. a small batch of vials processed in laboratory equipment. The problem of parameters regression is numerically complex and, if not well-conditioned, inaccurate. To improve the conditioning of this problem, Fissore et al.[100] re-formulated the DPE algorithm reducing the number of parameters to be estimated. They proposed to calculate the resistance to vapor flow (R_p), or equivalently the effective diffusivity k_1, from the initial slope of the pressure rise curve. This algorithm is commonly denoted as DPE+. By this way, the product temperature at the sublimation front remains the only parameter to be estimated, thus improving the conditioning of the regression problem. Fissore et al.[100] also observed that, independently of the type of algorithm used, the accuracy of the parameters estimation strongly depends on the duration of the PRT. More specifically, the optimal duration of a PRT is directly related to the time constant of the process:

$$\tau = \frac{VL_{dried}}{k_1 N_v A_v} \tag{10}$$

that is a function of the equipment size (i.e. volume of the chamber, V), the batch size or loading (i.e. number of vials, N_v), and the formulation (i.e. the effective diffusivity of vapor through the dried layer, k_1). If the PRT is shorter than τ, the dynamics of the process is not completely captured. By contrast, if the PRT is much longer than τ, the accuracy of estimations is poor because the problem of regression of the parameter of interest is ill-conditioned, particularly in the second part of primary drying.

The performance of the various PRT algorithms have been compared by Fissore et al.[100]. As shown in Figure 6, all the PRT algorithms underestimated the product temperature

after 8 h of drying, 5 h earlier than the time at which the Pirani-Baratron pressure ratio curve starts to decrease. This result was likely due to the bad-conditioning of the regression problem; in fact, the DPE+ algorithm, which is based on the estimation of only one parameter, gave accurate estimation till the onset time of the pressure ratio curve, i.e.13 h. Then, its estimations of the product temperature decreased because, as it is well known in the literature, the sublimating area is not anymore constant due to batch heterogeneity. As concerns the estimation of all the other parameters, e.g. rate of sublimation and heat transfer coefficient, PRA, DPE and DPE+ gave similar results, whereas MTM systematically underestimated these parameters.

All these algorithms were validated essentially for water-based formulation, but, in principle, they can also be used in presence of other organic solvents or mixtures of solvents at the eutectic composition. In the case of mixtures having a composition different from the eutectic, it is necessary to detect the partial pressure response of all the components of the mixture, as described by Fissore et al.[101]. To provide an example, Figure 7 shows the pressure response for a drug formulation containing a mixture of water and *tert*-butyl alcohol. The evolution of the total pressure was detected by a capacitive manometer, while that of the partial pressure of water was obtained through a laser spectrophotometer. Their difference gives the pressure response of the *tert*-butyl alcohol (graph B).

A limitation of the PRT method is that it requires a fast closing isolation valve between the drying chamber and condenser.[55] This is particularly true for industrial scale units, where the time necessary to close the isolation valve is of the same order of magnitude of the duration of the PRT. Nonetheless, this problem can in principle be overcome by including the dynamics of the isolation valve, as commonly occurs in the control practice of chemical processes[102]: Pisano et al.[103] included the dynamics of the pressure sensor into the DPE+ algorithm, thus improving the performance of DPE+ in the case of aggressive cycles or

full loading conditions, as in these conditions the increase of pressure is so fast that the dynamics of the pressure sensor is not anymore negligible.

Provided that the PRT parameters have been set appropriately, the accuracy of the PRT estimations might still be poor in the case of high-concentrated amorphous formulations.[56] This behavior is not completely clear, but it has been hypothesized that it is due to the partial re-adsorption of vapor over the surface of the dried product during a PRT, damping the increase in pressure. Another drawback of PRT is that it regularly perturbs the process and product dynamics, and this may be undesirable in a manufacturing cycle.

PRT can in principle be used also for monitoring the residual moisture *vs* time during secondary drying, as it will be discussed in a following section. To overcome some of the drawbacks of PRT, and in particular the increase (even if generally small) of the product temperature during the pressure rise and the necessity to frequently close the large valve in the chamber-condenser duct, other approaches have been proposed, shutting the valve connecting the vacuum pump to the condenser or the inert gas bleeding.[101] In principle they are very similar to PRT, as also in this case the system is perturbed and its response is analyzed to recover the values of the parameters of interest of the system model. In particular a test named Pressure Decrease Test (PDT) has been conceived and validated, bot for water and water-cosolvent mixtures[104]; it will be discussed in the followings, together with the Valvless Monitoring System, as it takes advantage of a similar modeling approach.

Other monitoring systems based on measurement of the sublimation flux

Besides software sensors based on temperature measure and PRTs, there are other classes of methods that have been proposed specifically for the monitoring of the sublimation flux. This information can then be combined with a mathematical model of the primary drying process to infer other parameters of interest, e.g. the product temperature at the sublimation front.

The Tunable Diode Laser Absorption Spectroscopy (TDLAS) is one of the ways proposed in the literature to measure the vapor flow rate released via sublimation during primary drying.[105] Gieseler et al.[106] proposed to install the TDLAS sensor directly in the duct connecting the drying chamber and condenser. There, TDLAS emits a beam of diode laser radiation which is partially absorbed by water molecules at a specified wavelength. The number of water molecules per unit volume is then determined by the amount of absorbed light at the wavelength specific of water absorption. If the diode laser beam is orientated at a given angle, different from the normal direction to the vapor flow, the absorption is shifted in wavelength or frequency with respect to that observed when the gas is at rest or the measurement is performed at an angle normal to the vapor flow. If the angle shift is known, the vapor flow rate is calculated from the Doppler-shifted absorption spectrum of water. Gieseler et al.[106] also compared the vapor flow rate as measured by TDLAS with that obtained by the gravimetric procedure in a lab scale apparatus. They observed that in the pilot scale apparatus the ratio between the two measurements was 1.02±0.06 for the freeze-drying of pure water and 0.96±0.05 for a 5% (w/w) mannitol solution, confirming that TDLAS can accurately estimate the mass flow rate of vapor through the duct. Its time integration can then be used to estimate mass of water separated via sublimation and, hence, the residual water content. The same authors also showed that TDLAS can be used to estimate the overall heat transfer coefficient of the vials used to carry out the lyophilization process. Of course, this estimation requires the knowledge of both sublimation rate and product temperature, and this last parameter needs to be measured independently, e.g. through thermocouples. Later, Schneid et al.[107] showed that TDLAS can also be used to monitor the average product temperature of the batch of vials. More specifically, they proposed to carry out a first lyophilization cycle using pure water, measuring the vapor flow rate through TDLAS and the product temperature by thermocouples. This test allows the determination of the heat transfer

coefficient of the vials. A rapid procedure for determination of the heat transfer coefficient using step changes in pressure set point has been also proposed by Kuu et al.[108]. Then, TDLAS is used to monitor the vapor flow rate of the target cycle that processes the drug formulation. Since the heat transfer coefficient of the vial is known, the TDLAS measurement of the vapor flow rate can be used to estimate the average product temperature of the batch of vials:

$$T_B = T_{shelf} - \frac{J_w}{K_v \Delta H_s} \tag{11}$$

where T_{shelf} is the shelf temperature as measured through thermocouples. Schneid et al.[107] observed that the average product temperature, as estimated by TDLAS for both sucrose- and mannitol-based formulations, was very close to that measured through thermocouples for central vials, within the range of 1-2°C. The TDLAS can also be employed for direct determination of product mass transfer resistance during a freeze-drying cycle; Kuu et al.[109] proposed three methods, using (i) the sublimation rate profile alone; (ii) the product temperature profile alone; or (iii) both of them: in the latter case the parameter can be determined without solving the complex heat and mass equations.

Schneid et al.[110] in addition demonstrated that TDLAS has some potential for monitoring also the vapor flow rate released via desorption during secondary drying, as it will be discussed in the following. Unfortunately, TDLAS is not compatible with all the freeze-dryers. In fact, its application as mass flow-meter requires that the duct connecting the drying chamber and condenser is long enough to allow the absorption measurement at an angle sufficiently different from the normal direction of the vapor flow. For example, Schneid et al.[107] needed to modify their freeze-dryers: the laboratory unit was outfitted with 0.22 m-long duct, while the pilot scale unit with a 1 m-long duct. Furthermore, if the mass flow rate of TDLAS is specified to agree within 6-7% vs. gravimetric determination, discrepancies of up to 25% have been observed when TDLAS is used on manufacturing units under aggressive

cycles.[55] This large error is likely due to the measurement of the fluid velocity which is necessary for the calculation of the mass flow rate. Besides, the TDLAS data analysis algorithm assumes an axi-symmetric velocity profile, and this assumption is not anymore true in presence of obstacles in the flow, e.g. the clean-in-place/sterilize-in-place piping. Furthermore, steam sterilization can produce some drift in the optical alignment of the sensor, requiring frequent regulation of the optical hardware.

The duct itself can be used as a measuring tool for the gas flow rate leaving the chamber[20], even if it is necessary to take into account also the stream composition. Thus a valid alternative to TDLAS for the measurement of the sublimation rate is the Valveless Monitoring System (VMS) described in Fissore et al.[101] and in Pisano et al.[111]. VMS correlates the pressure drop along the duct connecting the drying chamber and condenser with the mass flow rate of vapor evacuated from the drying chamber itself. Of course, its application requires a calibration curve of the equipment, defining the correlation between vapor flow rate and pressure drop, and two capacitive pressure sensors at the inlet and outlet of the duct which are commonly present in a freeze-dryer. Once the vapor flow rate is measured, if the heat transfer coefficient of the vial is known, the average product temperature of the batch can be estimated. VMS algorithm was demonstrated to give accurate estimations of sublimation rate and product temperature also for drug formulations containing both pure water[101] and mixtures of *tert*-butyl alcohol and water[111]. There might be perception that VMS requires highly specialized training, but this is not true: the VMS calibration is done prior to installation of the equipment and is valid for its entire life. Of course, this is true only if any structural changes are made into the duct.

Based on the same principle and partially on the same model equations is the PDT method, mentioned before, which analyzes the response to a variation in the inert flow rate, that is the reduction of the chamber pressure; generally, a stop of inert bleed is used, as in this

case it is not necessary to monitor accurately the mass flow controller on the bleed line.[104] With this method, that similarly to the VMS allows to determine, from the estimated sublimation flux, the average product temperature and the transfer parameters, thus, only a temperature decrease can occur, with no danger for the product (the only consequences may be a small increase in the drying time). It must be evidenced that the PDT method can be applied easily to large scale apparatus, as no large moving parts are involved, and as VMS does not require any additional hardware device to the normal equipment of production scale freeze dryers.

The use of a micro-balance is another way to monitor the sublimation rate of a single vial or a small number of vials. Various technological solutions were proposed in the literature. Bruttini et al.[112] developed a weighing device that measures the mass of a system made of the heating plate, the tray and the loaded vials. This device requires that freezing is performed separately, because the cryostat produces vibrations that can perturb the mass measurement. In a different realization, the installation of the weighing device implies the addition of a gap separating the heating source and the vials, which further limits the heat transfer efficiency between the equipment and the product being lyophilized[113]. Other technical solutions, e.g. the device commercialized by Martin Christ (Osterode am Harz, Germany), can solve most of these problems, but it can monitor the mass of only one vial. Furthermore, the monitored vial is customized and undergoes different heat transfer conditions with respect to those of the batch[114]. An important improvement in this direction was given by Carullo et al.[115]: their micro-balance can monitor up to 15 vials, having the same geometry of those of the batch. However, the monitored vials tend to receive more heat, with respect to the vials of the batch, because of radiation from the chamber side-walls. However, Barresi et al.[49] and Carullo and Vallan[116] showed that, if the monitored vials are shielded from side-radiation using an aluminum foil, or placing the other vials of the batch at

the side, the drying behavior of monitored ones becomes very similar to that observed for the rest of the batch. As already discussed for TDLAS and VMS, the sublimation rate as measured by the micro-balance can then be used to estimate the other parameters of interest.

There is another methodology that can in principle be used to estimate directly the vapor flow rate released via sublimation, even if it has not achieved much success: this technology is known as windmills: it was proposed to install a windmill to measure the flow from chamber and, thus, to monitor the sublimation rate, and eventually use this feedback for process control. In this case the chamber pressure would be controlled manipulating the vacuum device[117]. The sensors could be inserted in the duct connecting the chamber to the condenser, or in large industrial apparatus preferentially in an alternate path, and it is claimed that in this case it could be also employed selectively to identify completion of the process.

As the pressure in the chamber depends on the sublimation rate, its measure would be an indirect evaluation of the sublimation rate; actually, pressure in the chamber is generally directly controlled, either by an on-off control of the vacuum pump, especially in the small pilot-scale equipment in laboratory, or by controlled leakage. In this case, the signal of the mass flow controller was shown to be in close agreement with the temperature response, increasing when the latter decreases, and, thus, it offers an easy and cheap way to detect the end of the sublimation. It is surely a noninvasive tool, which can be installed upstream of the venting filter, suitable for routine monitoring of the cycles[118].

The reduction of the sublimation rate (and the consequent increase of the inert bleed flow) at the end of primary drying causes an increase in the condenser pressure: it has been proposed to use this variation, measured by a capacitive gauge placed in the condenser, to detect end of primary drying, but the pressure increase is generally very small, making difficult to clearly identify it.[53] In any case, the measure of the pressure difference between chamber and condenser might be used to estimate the flow in the duct, and, as discussed

before, in particular to detect conditions leading to chocked flow.

Other monitoring systems to detect end of primary drying, residual moisture content and other properties

The sublimation flux and the chamber pressure control method affect the composition of the gas in the chamber: during primary drying the gas is mostly composed by water (or by the used solvents, in the case of water-cosolvents systems), but at the end of primary drying, when sublimation slows down, the inert gases prevail. Thus, monitoring the gas composition in the chamber allows to detect the end of primary drying.

The Vapor Sampling Condensing (or trap method) was one of the early method used for measuring the partial vapor pressure; it was described by Kan[119] and is today sometimes still used in small apparatus for research[120],[121].

A simpler and more reliable method is that called ”comparative pressure measurement” suggested by Nail early in 1980[122],[64] and still considered one of the cheapest and most reliable methods to detect the end of primary drying, also in industrial apparatus. It takes advantage of the dependence of the signal of a thermal conductivity gauge, like the Pirani gauge, on the gas type and, in case of mixtures, like water and inert gas, on the composition. This would be a strong limitation for simple pressure monitoring, because the chamber gas composition differs in different cycles depending on set up, loading and product features[122], and, as a consequence, this type of pressure sensor, which is quite cheap, but has also a lower accuracy than the capacitance manometer, is used only in low price lab equipment. The use of the ratio of the pressure signals given by the two gauges, the thermal conductivity and the capacitive ones, that approaches unity (offset point) at the end of the primary drying as the Pirani is generally calibrated for air, is quite reliable, because it eliminates the possible effect of a variation of the total pressure, and also quite sensitive. In

fact, as the partial pressure of water falls when the sublimation rates strongly decreases, but it reduces below detection limits only when the sublimation flux is extremely low, the method allows to detect the last vials that complete primary drying: thus, it offers a safe-side indication even if the estimated drying time may be slightly longer than real as the change in the chamber atmosphere is also influenced by geometry, condenser operation and pumping rate.

As the point where the signal starts decreasing with a large slope (onset point) corresponds to the time at which a significant fraction of vials have completed the sublimation phase[121] the analysis of the shape of the decreasing curve can offer information on the intra-vial heterogeneity: in particular the difference between the onset and offset time has been used to this purpose, also to evidence the influence of the freezing protocol[40], even if this parameter is influenced in a complex way by batch size and operating conditions.[123],[124]

Misleading signals, with prolonged drying times, can be obtained in case of abnormal configuration (for example vials that fall off the shelf and thus dry much more slowly, keeping relatively high the signal even when the batch has completed drying.[64]

A possible limitation of the method is the concern about the possibility to steam sterilize the Pirani, and its use in presence of organic solvents, which can give flammable mixtures in air, for the presence of the hot spot in the sensor, which must be in contact with the gas. The first point is still controversial, and probably depends on the model adopted[55], even if in some cases it was reported that the Pirani could withstand sterilization without problems[53]; new models using different materials for the filament (nickel or platinum rather than the standard tungsten) can show better performances to this respect. Frequent recalibration, or even replacement of the sensors, that, as said, is quite cheap, could be another possibility. New models equipped with a stainless-steel shield to protect against condensation, and using a pulsed mode of operation, have been developed, which also guarantee higher

signal resolution, extended range of measurement and better long-term stability. As concerns the use with organic solvents, the Pirani must be turned off during the operation at ambient pressure, and when the vacuum is broken, but as the normal low pressure operating conditions are out of the flammability zone, it can be safely used during drying.

Alternatively to the Pirani gauge, a dew point sensor can be used to measure the relative humidity and, thus, the gas composition in chamber: the use of this type of device, which would allow a greater sensitivity and a more accurate detection of end point was proposed since the end of the Sixties, but its performances were deeply investigated only twenty years later[121],[125]. The sensor exploits the variation of the capacity of a thin film of aluminum oxide due to moisture, and its sensitivity allowed to detect the presence of less than 1% of vials in the batch with residual ice. As already mentioned, the device measuring the moisture content of the gas predicts a significantly longer drying time that those based on temperature measurement, and the difference is also related to the batch size, largely increasing with it. This is probably a consequence of their higher sensitivity, and moisture desorption from the chamber wall may also have a role; slow desorption of moisture from the probe was also investigated, but Roy and Pikal[121] estimated that this was not affecting the sensor performance, that was also tested in an industrial scale freeze-drier. Other models of moisture sensors were also made commercially available, based on the same capacitive principle, in recent year: these sensors were tested by several researchers, also in comparison with other types of device, like the quadrupole mass spectrometer (QMS) or the cold plasma ionization device[126]-[130],[49] for end point detection. Qualitatively, the mentioned methods and the comparative pressure measurement give a similar response, and a similar value for the offset-onset difference, but the cold plasma sensor and the Pirani/Baratron allowed the best determination of end point, even if the signal of the second one was more noisy, probably as a consequence of gas injection for pressure regulation, while the QMS showed problems with

the stability of the baseline, and the moisture sensor not always allowed a clear identification of the end point[130],[49]. In addition it must be remarked that the response time of the moisture sensor is very long at low pressure, probably as a consequence of diffusion and desorption phenomena, which makes this sensor not suitable for dynamic response analysis.

Genin et al.[126],[127] developed a procedure based on standard law for mass transfer for the estimation of the residual water content in the product at any time, and made use of a mathematical function which reaches zero value at the sublimation end-point to improve the method.

The cold plasma ionization device seems to have the highest sensitivity. It is based on inductive coupled plasma/optical emission spectroscopy: by means of radio-frequency it creates cold plasma in a quartz tube under vacuum, and the light emitted by the plasma is characteristic of the gas present in the plasma. A measure of the humidity can thus be obtained in real time analyzing the optical spectrum. It has a good potential for application also in industrial scale apparatus, being steam sterilizable and reproducible, even if it presents some drawbacks: the uncertainty on the final point determination, the problem of calibration and the dependence of the response on the probe location[131],[130]. The main concern is related to degradation of products susceptible to free radical oxidation, as a consequence of the ionization of the gas present in the chamber: Patel et al.[53] showed that substantial decomposition can occur, for example, with human growth hormone, proposing to move the sensor from the chamber to the duct to reduce the problem: it was shown that the sensor was sensible to the gas composition in both locations, even if this may require some modifications to the existing driers.

Absorption spectroscopy is another technique available for measuring the average composition of the gas in the chamber: a tunable diode laser is generally employed. The use of TDLAS has been already discussed to measure the sublimation flow, from composition,

temperature and velocity of the gases in the duct. A simpler configuration is also possible, with a single laser beam crossing the chamber (or even the duct), if the purpose is simply that of determining the moisture concentration and, thus, the end point, taking advantage of the drop observable when primary drying is almost finished[132],[52],[101],[105]. Such a set-up, in the chamber, would also allow to obtain the pressure rise response of the water vapor, useful in case of water-organic solvent mixtures, as previously discussed. To provide an example, Figure 8 shows the evolution of water concentration within the drying chamber as detected by the TDLAS sensor. It can be observed that its signal started to decrease after approximately 15 h of drying, which corresponds to the onset time of the pressure ratio curve. At that time, the vapor flow rate as estimated by PRT is almost zero and product temperature sharply increased indicating that primary drying was almost completed.

Mass spectrometry is certainly the technique with the highest sensibility, but notwithstanding the fact that its use (employing a radio frequency mass-spectrometer with rapid response) was proposed since 1962 by Kan[119], who showed that it could work even in absence of inert bleeding, and the successive works where a quadrupole mass spectrometrer was employed, to monitor both primary and secondary drying[133],[2],[64],[58],[134],[135],[51],[129],[54] since now it has not been widely employed. The reasons for that rely on the difficulty in obtaining quantitative measurements, as calibration is difficult and must be repeated in each run, and the baseline is not very stable, as already noted, even if Jennings[133] suggested the use of a capacitance manometer to make the calibration. It is true that significant information can be obtained also simply monitoring the time evolution of the ionic current corresponding to the fragment of mass 18 (i.e. water), divided by the total pressure measured by the instrument, as suggested again by Jennings, but the information that can be obtained relatively to the end of primary drying is equivalent to that of much simpler and low-price sensors. In fact, mass spectrometer was extremely expensive, and previous models had also large

volumes that made difficult their use. Recently, the interest for this device greatly increased for quality control, and some manufactures routinely install it on production freeze-driers. New models are small and portable, and are now considered precise, accurate, fast and cost effective [136]. The QMS has some other advantages: it can be used also with organic solvents, allowing to monitor both water and solvent flux. In addition, it is suitable also for secondary drying monitoring, where a few methods are available, and its performance is not compromised by single vial effects, and it is sensible also to cleaning solvents, outgassing of closures and elastomeric components. Finally, it can be used as leak detector (employing helium burst to the external part of freeze-drier) and can monitor oxygen partial pressure which is relevant for oxidation sensitive products[137][51][52]. The mass spectrometer is not sterilizable, but similarly to the moisture sensor, it can be separated by a sterile gas filter.

Finally, it must be considered that correct location of all the sensors discussed is very important; in fact, the measurements obtained from both devices that sample the chamber atmosphere, generally in a peripherical position, and those that make an integral measurement along a line, as the TDLAS, can be affected by non-uniformity of the gas composition. CFD simulations have evidenced that in case of pressure control by inert bleeding strong concentration gradients can occur[138],[98].

All the methods described in the first part of this section are based on the measure of the gas composition, that changes as a consequence of the sublimation rate, and thus monitor the average behavior of the batch. A different approach is that of measuring directly some properties of the product inside the single vials, either in an invasive or non-invasive way. The insertion of a probe in the product presents many problems, already discussed in the case of a temperature-measuring probe, but, in any case, the representativeness of the monitored sample must be taken into account, or a multi-point measuring system must be realized to take into account the heterogeneity of the batch.

Monitoring of electric properties, and in particular of the product resistance (or of the impedance), has been proposed since long time ago[139]. It is applicable mainly to substances that show a sharp eutectic melting, as in this case there is a very large variation of the resistance when the product approaches the limit temperature, even if by impedance also measurements variations of the mobility of some species in non-crystalline matrixes can be evidenced. The objective is to prevent melting during primary drying, conducting the step at the maximum allowable rate, monitoring these electrical variables as an alternative to temperature measurements. Notwithstanding some reliability problem, the necessity to insert the electrodes in the vial and, sometimes, the difficulties in interpreting the results, the method is used in laboratory for process development, it is commercially available as an option in pilot scale apparatus sold by various manufacturers, and it is present in several patents about freeze-drying control[10][139]-[143],[2].

Capacitive sensors are applicable to both primary and secondary drying steps[144],[145]. In particular, a non-invasive tool based on impedance spectroscopy has been recently investigated: it has the electrodes on the outer surface of the vial, and measures the pseudo-relaxation process associated with the interfacial polarization of the glass wall, allowing to measure rate of drying, and to detect microcollapse and the end of primary drying[146]. These systems can measure a large volume, or the whole sample, and not simply a small portion, as is the case of the NIR or Raman sensors.

Near Infrared (NIR) and Raman spectroscopy, on the other side, are getting a lot of interest at least at lab scale and for process development because, especially if combined with other sensors, they allow precise determination of the end of both primary and secondary drying stages, the direct measurement of the moisture during different dying stages and to monitor physical transformation of the different components (and protein physical stability) in all the freeze drying process, including freezing[63],[147]-[149]. Early works adopting NIR in-line

used invasive probes, but they evidenced the potentiality of the method to monitor changes in the physical state of the product and the results showed a good agreement between NIR spectroscopy and product temperature monitoring as concerns, in particular, the freezing step and the end of primary drying.[150],[151],[152] In the previous case the single fiber reflectance probe of the FT-NIR probe was positioned inside the vial together with a thermocouple.[151] Wiggenhorn et al.[51] positioned the NIR sensor on the shelf inside the drying chamber, directly fitted with the probe tube to the outer wall of the vial, showing that it was possible to monitor the process using NIR in a non-invasive way, reducing the concern for sterility, without constrains on vial type and modification of the filling volume, and not disturbing significantly the drying kinetics (even if some additional heating effect was present, especially in the early prototypes).

Non-invasive, in-line and real-time analysis of the lyophilization process by means of Raman spectroscopy (coupled with at-line NIR spectroscopy and X-Ray powder diffraction) was applied by De Beer et al.[153] who placed a fiber-optic non-contact probe above the freeze-dried product. Raman spectroscopy allowed to monitor physical phenomena during freezing (i.e. ice and mannitol crystallization), end points of freezing and primary drying, the mannitol solid state and resulted applicable to the whole process. It must be said anyway that if NIR proved to be much more sensitive to monitor sublimation and, thus, to detect end of primary drying, because water and ice produce very weak signals in Raman spectra, but strong absorption in NIR spectra (and, for this reason, generally the signal from ice overwhelms that of the other components). The potentiality of Raman spectroscopy was also confirmed by Romero Torres et al.[154] for the freezing step.

As previous work evidenced the complementarity of NIR and Raman spectroscopy, especially during primary drying, NIR and Raman spectroscopy were employed simultaneously in-line[63],[155]. In this case the NIR and Raman probes must be placed on two

different vials to avoid that the Raman signal is saturated by the reflected NIR beam (during primary drying). The position of the probe is important in order to obtain reliable measurements: it was suggested to locate the Raman probe above the product and the NIR probe at the bottom of the sidewall of the vial[155],[52]. Anyway, it was shown that also NIR spectroscopy allows in-line monitoring of the behavior of all components of a multi-component system during the whole process, including freezing[156].

Monitoring of the secondary drying

In the secondary drying stage, the monitoring system has to track the residual amount of moisture in the product, aiming to point out the ending point of this stage. Generally, a very low value of residual moisture is desired, even less than 1%: in any case, the target value is a characteristic of the product being processed[157].

Sampling of vial from the drying chamber, through a sample thief placed on the door, followed by the measurement of the residual moisture (using Karl Fischer titration or other methods), is the usual way of monitoring the secondary drying stage[158], in particular in lab-scale units, although it can be used also in industrial scale apparatus, provided that the extraction of samples from the chamber is feasible.

Few devices were proposed to monitor in-line the secondary drying stage. Some of them, suitable for process development, measure directly the residual amount of solvent in the product. Dielectric measurements have been proposed also for detection of secondary drying endpoint[159],[145],[160] or, at least, to find the optimal residual moisture (which corresponds to the minimum of intramolecular mobility)[144]: the electrodes can be placed outside the vial to reduce the interference with the process.

NIR spectroscopy can also be used to detect the residual moisture of the sample; a method for multipoint detection, which makes possible the simultaneous analysis of several

vials with the same device, has also been proposed and validated[161],[162]. It correlates well with results from other methods, but it must be evidenced that it always underestimates the residual moisture (with respect to off-line Karl Fischer measurements) due to the incomplete penetration of the NIR-waves into the core of the sample[51]. NIR (and Raman) spectroscopy, on the other hand, allow monitoring also other important characteristics of the product, like protein unfolding (and generically protein conformational stability) and liposome structural integrity[148],[163],[164].

Other devices allow pointing out the ending point of the process, measuring the solvent vapor concentration in the drying chamber, or allow to track the dynamics of the solvent desorption rate. The cold plasma ionization device[131] and the mass spectrometer, already described for primary drying monitoring, are suitable also to detect the ending point of the secondary drying stage using the measurement of the water concentration in the chamber.

The Tunable Diode Laser Absorption Spectroscopy (TDLAS) was also proposed to monitor the secondary drying stage[165],[110], although the very low concentration of solvent vapor in the chamber, and the very low value of solvent flow rate, may seriously jeopardize the accuracy of the obtained values. In any case, once the solvent flux is calculated, it can be integrated in time, thus obtaining the solvent loss in the product and, if the residual amount of solvent at the beginning of the secondary drying process is known, the residual amount of solvent vs. time can be monitored. As an alternative, Schneid[166] and Schneid et al.[110] related the measured values of desorption rate, obtained through TDLAS, with the residual amount of moisture, obtained from sampling and analyzing the product in some vials during the process.

The pressure rise test was also proposed to monitor the secondary drying stage, as said before. In this case, the solvent flux is calculated from the rate of pressure increase in the chamber at the beginning of the test as described in Eq. (9). From the value of the solvent flux

calculated from the k-th PRT ($J_{w,k}$) it is possible to calculate the solvent loss (Δw_k) in the time interval between two consecutive PRTs (Δt_k):

$$\Delta w_k = J_{w,k} \Delta t_k \tag{12}$$

In case the value of the residual moisture is known at a certain reference time t_0, the residual solvent at any time t_i can be easily calculated from the summation of all the weight loss Δw_k occurring in the intervals between the PRTs from t_0 to t_i[167],[168]. As an alternative, Willemer[58] related the pressure increase measured during the PRT to the residual moisture, to determine the ending point of secondary drying. This approach was pursued by Oetjen[169]: he used the values of two successive measurements of desorption rate to extrapolate when the solvent flux reaches a small value, but he also pointed out that this approach fails in the last part of this stage, as it is very simplified. Fissore et al.[170]-[172] proposed a method based on the PRT and able to estimate: (i) the amount of residual solvent in the product when the secondary drying is started, (ii) the dynamics of the residual moisture during the secondary drying, and (iii) the time necessary to get the target value of moisture in the final product. This was obtained without extracting any sample from the chamber, without using (expensive) sensors to monitor product moisture during the test, and without carrying out any experimental investigation to get empirical correlations relating the solvent desorption rate to the residual moisture. Details of the algorithm can be found in Fissore et al.[170]: essentially, it is based on a mathematical model describing the variation with time of the residual moisture (eqs. (3) or (4) are used to model the kinetics of solvent desorption), and on a least-square algorithm that estimates the solvent concentration at the beginning of the secondary drying stage and the kinetic constant looking for the best fit between the measured and the calculated values of the solvent flow rate at each PRT. Figure 9 shows an example of the results obtained by this way, in terms of both the solvent desorption rate (measured with the pressure rise test) and of the residual moisture in the product (measured through Karl Fischer titration of

sampled vials), pointing out the adequacy of the proposed method.

Conclusions

PAT systems, which include the tools for process design and optimization, monitoring and control, are essential to allow maintaining the quality level at the target value; in this article the main systems and device proposed for process monitoring have been reviewed and critically analyzed, grouping them on the base of the physical principle utilized. Comparison of the different technologies considering their applicability to industrial scale have been already reported[131],[52], but here some further considerations have been summarized, considering potential use and limitations of the technologies described, and the transferability of the results obtained with them through scales.

To be utilized at production scale, the PATs must require minimal modification of the existing apparatus, sustain steam sterilization and be compatible with automatic loading systems and with final stoppering. These requirements *de facto* strongly limits the application of almost all the single-vial methods. They are particularly useful in the early stage of process development, and to get understanding of the phenomena occurring and of possible constrains, especially when low sample volumes are available, or for analysis of vial to vial variations. A comparative evaluation of the different techniques can be made considering the possibility to use them non-invasively and the level of competence required to employ them. NIR and Raman probes are still at the research stage: they are very powerful but the analysis of the large amount of data is quite complex. Conductivity techniques are invasive, because the electrodes are immersed in the product, while capacitive methods may be not simple to interpret, but a commercial non-invasive device (Lyosense™) is already available[145].

The use of scales and microbalances has also strong limitations: a commercial device is available, holding a single vial, and a different one suitable for a small cluster of vial has

been investigated, but it has been evidenced that the drying kinetics measured by this way is always faster than that of the real batch, because of the enhanced heat transfer due to radiation unless special expedients are used. This device can be useful mainly for determination of cake resistance.

Measuring of heat flux is interesting, as it has some potential also for large scale apparatus. It has been already incorporated in a PAT tools (LyoPAT^{TM}) in microfreeze-drier and at pilot scale[173] and, in principle, it is possible to insert several sensors in different position to take into account batch heterogeneity.

Product temperature measurement is one of the oldest methods and still probably the most employed one at pilot and lab scale, for its simplicity and low cost, notwithstanding the well known limits and the fact that is an invasive one. Advantages and disadvantages of thermocouples, thermoresistors and TEMPRIS have been discussed, and are mainly related to the wire connections of thermocouples and TRD, and to the size of TRD and especially TEMPRIS. Recent developments, even if still at prototype level, for thermocouples, with the possibility of getting wireless devices and sputtered (non-invasive) sensors, and their use as soft-sensor allowing to monitor the whole status of the sample, make them very promising for application at pilot and even at industrial scale. In addition, they make possible the monitoring of the different parts of the batch and, potentially, a control logic that takes into account the batch non-uniformity[66], overcoming the big limits of all the single-vial PAT tools, that is the representativeness of the selected vial.

Some of the PAT tools previously mentioned, like the Trough-Vial Impedance Spectroscopy[174] or Raman spectroscopy[175] are particularly suitable for microscale and mini-pilot studies: these devices utilize samples of different size, from microtitre plates to single vial or microclusters of vials. These devices are of great interest for process development, but the realization of reliable mini-piloting studies is still a big challenge, because there is no

single PAT technology for freeze drying that can be implemented reliably trough all scales, and the differences in process characteristics related to scale change (scale up problems) must be accounted for. The single vial or small cluster is the size that reduces the impact on product in relation to the container geometry; thus, the two non-invasive PAT tools available for this scale quoted above have good potential[174].

The batch monitoring methods are those that generally may be applicable both at pilot and production scale and, thus, whose results may potentially be transferred from one scale to the other, even if scale up is still an issue, as the influence of non-uniformity on the measured average variable may be different when the batch size changes[176],[177]. Also the leak rate can significantly change with scale, and this variable can significantly influence the output of the comparative pressure measurement, of the pressure rate tests and of the other sensors that measure the moisture content in the chamber gas. Actually, the ratio of the Pirani/Baratron measurement is still considered the PAT with the best performance/cost ratio, and is commonly employed even at large scales, notwithstanding some concerns related to sterilizability and performances of the Pirani gauge. Dew point and cold plasma detectors are commercially available PATs, more sensible (especially the cold plasma one), than the Pirani/Baratron, but substantially equivalent and more expensive. In addition, they have some drawbacks, respectively the slow response and the ionization of the chamber gas with consequent potential product degradation. The dew point monitor has been incorporated as a PAT tool into the laboratory scale freeze-dryers of various manufacturers, but both devices have been tested or have no limitation to use in large equipment, even if it must be taken into account that response depends on probe location.

Quadrupole mass spectrometers, even if very expensive, are now commercially available in compact and even portable devices; they resulted not very interesting in research, but are often installed in industrial apparatus for quality control. A manufacture has developed

a PAT system based on QMS (LYOPLUS™) that is standard installation in production equipment.

All the devices measuring the moisture content mentioned before are utilized for detection of the end of primary drying but, as said, they all gave a more or less similar response curve characterized by an onset and offset: the drying time is not well identified, and the selection of the correct value is still a question of debate, even if the midpoint is frequently proposed[53]. The offset point is certainly the most conservative, and should correspond to the end of drying of the last vials, but also this value is not always easy to identify for the drift in the baseline of the sensor, or because the desorption rate from the fraction of dried material and from the chamber walls can have a value comparable to the value of the sublimation rate at the end of the primary drying; for this reason, special algorithms were proposed. The SEP(t) function has been proposed for the moisture sensor on the basis of an inspectional analysis[126]: it uses the values of the total and partial pressure in the chamber and of the partial pressure in the condenser. The EPD algorithm[123],[124] couples the measurement of sublimation rate and a mathematical model that predicts the evolution of the moving front position. In particular, EPD regularly estimates the fraction of water mass already separated (calculated by the time integration of the solvent flow rate) and checks if the variation of the sublimated mass of water with respect to the total one is lower than a desired value:

$$\left(\frac{m_{sub}\left(t_j\right)-m_{sub}\left(t_{j-1}\right)}{t_j-t_{j-1}}\right)\frac{1}{m}\leq\alpha \qquad (13)$$

The PAT tools which measure the sublimation rate, the TDLAS and the various PRTs are suitable for pilot and large scale apparatus, allowing to detect the sublimation rate during the primary (and secondary) drying in addition to the end point. TDLAS is particularly interesting as it allows continuous monitoring in a noninvasive way, but is has to be installed in a new

equipment, as retrofitting is problematic. At production scale anyway, it is still considered a developing technology, because calibration may be problematic for the presence of CIP/SIP piping and nozzles and discrepancies up to 25% (versus gravimetric measurement) are observed in primary drying (while at the authors knowledge it has still to be validated at industrial scale for secondary drying). In addition, there are some concerns about maintenance, especially for the thermal stresses related to sterilization, which can cause drift in optical alignment. At lab scale performances are better, and discrepancies in estimation of total water removed are specified to agree within 7%[55].

Pressure rise tests are commonly employed also at production scale to verify the end of primary drying, as they require only to close the valve between the chamber and the duct. They can be adopted during primary drying to determine product temperature and sublimation rate, but the accuracy of the estimation depends on the model adopted. Various approaches were proposed and sometimes patented and are commercially available, like the MTM, the BTM and the DPE, only as monitoring tool or as a part of more sophisticated tools for cycle development or process control (as SMART Freeze-Drier™ technology[178],[168], Thermodynamic Lyophilization Control, Lyometrics™ or LyoDriver[179],[180]). Some of them are available also at industrial scale, but their use here is limited for the risk to jeopardize product quality in the batch (in correspondence of the pressure rise there is also a temperature increase in the product, because increases the heat transfer from shelf to vials) and for technical reasons related to the valve size. For larger ducts, mushroom valves are used instead of butterfly valves, and their slow closure makes PRT analysis more complex, even if suitable; frequent closure of large valves is anyway not recommendable. To overcome these limitations a “valvless monitoring system”, that is a PAT tool without moving elements, using only pressure gauges and a mass flow meter connected with the bleeding system, has been proposed, suitable both for water and water-cosolvent systems[101],[111].

Dew point, cold plasma sensors and QMS, together with TDLAS and PRTs, can be employed also for residual moisture analysis, and detection of end of secondary drying, but require specific calibration; the current limit of previous systems is that they do not allow the evaluation of the residual batch moisture with a unique calibration not dependent on the formulation. The systems based on the integration of the desorption flux could be a solution, but the accuracy is related to the accuracy of the single measurements, and this may be low for low flow rate (and errors are added up). The method using a soft-sensor, with one of the sensors to estimate the vapor flux in chamber, seems the most promising, as it does not require any preliminary experiment to determine the relationship between desorption rate and residual moisture; also the use of the pressure rise test, which is the most economical way to measure the desorption rate, is very safe in secondary drying, as no significant temperature increase is observed. In addition, few closures are requested and slow closing time is not an issue as pressure increase is almost linear and quite slow, and closure of the connecting valve is a normal practice at the end of the cycle.

To conclude, it may be interesting to directly compare the performances of many of the sensors analyzed. Several experiments in apparatus equipped with multiple sensors have been reported in literature, and the results show the monitoring response during the freeze-drying cycle; many have been commented in detail in a previous review[54], and the most relevant are summarized in Table 1.

Anyway it must be considered, especially in the process development stage, that the information supplied by different PAT tools are often complementary (see for example Ref. [63] and [56]), while in production the use of multiple sensors can improve the accuracy, even if an algorithm, or a procedure, must be defined for data reconciliation, and redundance improve reliability. Finally, it can be mentioned that in commercial manufacturing not only monitoring is important, but data are recorded and retrieved for continuous process

verification (ICH Q8 - R2)[55]; the monitoring output of PAT sensors can be added to those that must be obligatorily recorded.

Acknowledgement

Part of the content of the paper is the synthesis of several years of research activity of the team at Politecnico di Torino, and of the cooperation with various Italian and foreign groups. The contribution of the colleagues Alberto Vallan, Sabrina Grassini and Marco Parvis, and of former Ph.D. students (in chronological order): Salvatore Velardi, Valeria Rasetto, Serena Bosca, and Irene Oddone from Politecnico di Torino, is greatly acknowledged.

List of symbols

A_v	cross section area of the vial, m^2
a	specific surface of the dried product, $m^2\ kg^{-1}_{\text{dried product}}$
C_s	residual moisture, $kg_{\text{water}}\ kg^{-1}_{\text{dried product}}$
$C_{s,eq}$	weight fraction of sorbed water in the solid that would be in local equilibrium with the partial pressure of water in the drying chamber, $kg_{\text{water}}\ kg^{-1}_{\text{dried product}}$
$\mathbf{f}$	vectorial function giving the derivatives of the state
h	state space equation of the measured variable
ΔH_s	enthalpy of ice sublimation, $J\ kg^{-1}$
J_q	heat flux to the product, $W\ m^{-2}$
J_w	mass flux, $kg\ s^{-1}m^{-2}$
$J_{w,k}$	mass flux calculate from the k-th pressure rise test, $kg\ s^{-1}m^{-2}$
$\mathbf{K}$	soft-sensor gain
K_v	overall heat transfer coefficient between the heating fluid and the product at the

	vial bottom, W $m^{-2}K^{-1}$
k_d	kinetic constant of the desorption rate, $kg^{-1}_{dried\ product}\ s^{-1}\ m^{-2}$
k_1	effective diffusivity of vapor through the dried layer, m^2s^{-1}
L	thickness of the product, m
L_{dried}	thickness of the dried product, m
M_w	molecular weight of water, g mol^{-1}
m	mass of ice in the vial, kg
m_{sub}	mass of solvent sublimated, kg
N_v	number of vials
P_c	chamber pressure, Pa
$p_{w,c}$	water vapor partial pressure in the drying chamber, Pa
$p_{w,i}$	water vapor partial pressure at the interface of sublimation, Pa
R	ideal gas constant, J mol^{-1} K^{-1}
R_p	resistance of the dried product to vapor flow, m s^{-1}
r_d	water desorption rate, $kg_{water}\ kg^{-1}_{dried\ product}\ s^{-1}$
T_B	product temperature at the vial bottom, K
T_i	product temperature at the interface of sublimation, K
T_{gas}	temperature of the gas inside the drying chamber, K
T_{shelf}	heating shelf temperature, K
t_{drying}	time required to complete the ice sublimation, s
t	time, s
Δt_k	time interval between two consecutive pressure rise tests, s
V	volume of the drying chamber, m^3

Δw_k	solvent loss between two consecutive pressure rise tests, kg

Greeks

α	parameter used to identify the ending point of the primary drying stage (eq. (13)), s^{-1}
ρ_{dried}	density of the dried product, kg m^{-3}
ρ_{frozen}	density of the frozen product, kg m^{-3}
τ	time constant of the process, s

Superscripts

^	soft-sensor estimate

Abbreviations

BTM	Barometric Temperature Measurement
DPE	Dynamic Parameters Estimation
DPR	Dynamic Pressure Rise
MTM	Manometric Temperature Measurement
PDT	Pressure Decrease Test
PRA	Pressure Rise Analysis
PRT	Pressure Rise Test
QMS	Quadrupole Mass Spectrometer
TDLAS	Tunable Diode Laser Absorption Spectroscopy

VMS Valveless Monitoring System

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List of Figures

Figure 1. Freeze-drying of a 5% by weight sucrose solution, processed in glass tubing vials (ISO 8362-1 8R), T_{shelf} = -20°C, P_c = 10 Pa. Graph (**A**): Product temperature at the bottom of a vial in the center of the shelf (solid line) and at the edge of the shelf (dashed line) measured by T-type miniature thermocouples. Graph (**B**): ratio between Pirani and Baratron pressure measurement. (Data taken from Ref. [66], and used with permission.)

Figure 2. Values of K_v obtained from the four temperature measurements in the test with a 5% by weight sucrose solution, processed in glass tubing vials (ISO 8362-1 8R), T_{shelf} = -20°C, P_c = 10 Pa. The value measured through the gravimetric test is also shown (dashed line), with the uncertainty range (solid lines).

Figure 3. R_p *vs* L_{dried} calculated using the various temperature measurements (lines) and the calculated mean values (symbols) of the parameters expressing the dependence of R_p on L_{dried} for the 5% by weight sucrose solution.

Figure 4. Graph **A**: Product temperature at the vial bottom (—: thermocouple, ○: soft-sensor). Graph **B**: Evolution of the sublimation interface calculated by the soft-sensor. Graph **C**: Ratio between Baratron and Pirani pressure gauges signals. Data refer to the freeze-drying of a 5% (w/w) sucrose aqueous solution, processed in glass tubing vials (ISO 8362-1); primary drying stage is carried out at T_{shelf} = -24.5°C and P_c = 5 Pa. (Data taken from Ref. [80], and used with permission.)

Figure 5. Example of pressure rise curve as observed for the freeze-drying of a 5% (w/w) sucrose solution after 3 h of primary drying at -10 °C and 10 Pa.

Figure 6. Example of PRT outcomes as obtained through various algorithms, (◊) MTM, (Δ) PRA, (□) DPE, and (■) DPE+. Evolution of (**A**) Pirani-Baratron pressure ratio, (**B**) shelf temperature, (**C**) product temperature at the sublimation front, (**D**) rate of sublimation, and (**E**) heat transfer coefficient. Comparison was done at constant duration of the PRT, i.e. 30 s. (Figure taken from Ref. [100], and reproduced with permission.)

Figure 7. Example of pressure rise curve as observed for a drug formulation containing 95% (v/v) of water and 5% (v/v) of *tert*-butyl alcohol. (**A**) Evolution of (●) the total pressure as measured through a capacitive manometer and of (○) the partial pressure of water as observed through a laser spectrophotometer. (**B**) The partial pressure of *tert*-butyl alcohol vs. time as calculated from the two signals shown in graph **A**.

Figure 8. Example of freeze-drying cycle of a 5% (w/w) sucrose solution, in vials, carried out in a special pilot scale freeze-dyer: LyoBeta 25 by Telstar (Terrassa, Spain) equipped with LyoDriver and a tunable diode laser (LaserGas II SP Monitor, Neomonitors, Skedsmokorse, Norway) installed within the drying chamber. Evolution of (**A**) product temperature as observed through thermocouples (solid line) and vapor flow rate as estimated by PRT (DPE+) (symbols), (**B**) water concentration as detected though the TDLAS sensor (with the beam crossing longitudinally the chamber) and (**C**) pressure ratio curve. Primary drying was carried out -10 °C and 10 Pa.

Figure 9 Comparison between calculated (lines) and measured (symbols) values of

desorption rate (graph **A**) and residual moisture (graph **B**), when T_{shelf} = 30°C and P_c = 5 Pa. (Data taken form Ref. [172], and used with permission.)

Table 1 – Comparison between different PAT tools in literature

Authors	ref	PAT tools compared
Nail & Johnson (1991) [reproduced by Ref. [52]	[64]	Comparative pressure measurement, Product T Pirani pressure, Residual gas analyzer
Mayeresse et al., 2007	[131]	Lyotrack, Microbalance (Christ), Comparative pressure measurement
Schneid & Gieseler, 2008	[61]	TEMPRIS, Thermocouples
Patel et al., 2009	[53]	Comparative pressure measurement, Pirani pressure, Dew point sensor (General Eastern), Lyotrack, TDLAS, PRT (MTM), Product T, Gravimetry + Karl Fischer [
De Beer et al, 2009	[63]	TEMPRIS (various locations), Lyotrack, NIR and Raman probes
Schneid et al., 2009	[107]	TEMPRIS, PRT (MTM), Thermocouples
Hottot et al., 2009	[130]	Pirani gauge, Dew point sensor (Panametrics), Microbalance (Christ), Lyotrack, Thermocouples
Barresi et al, 2009	[49]	Lyobalance, Thermocouples, PRT (DPE), Comparative pressure measurement, Dew point sensor (Panametrics), Residual gas analyzer (QMS)
Schneid et al., 2011	[110]	TDLAS (secondary drying), Karl Fischer

Figure 1

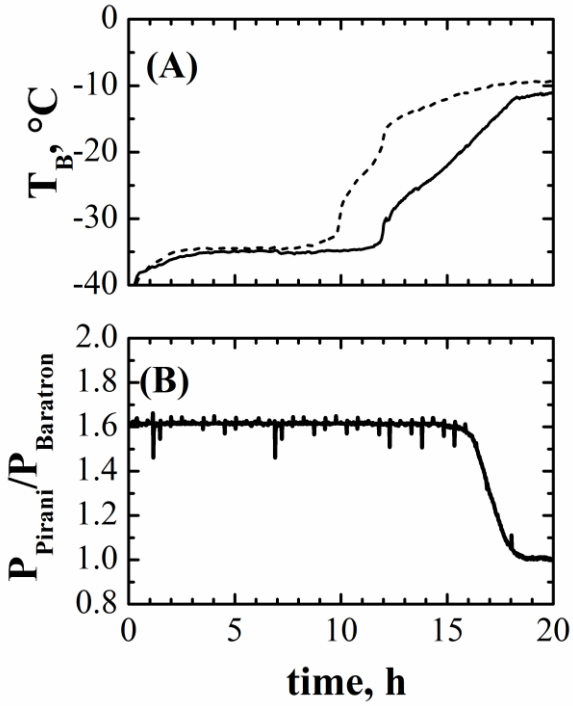

(A)
(B)
T_B, °C
$P_{Pirani}/P_{Baratron}$
time, h
0
-10
-20
-30
-40
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0
5
10
15
20

Figure 2

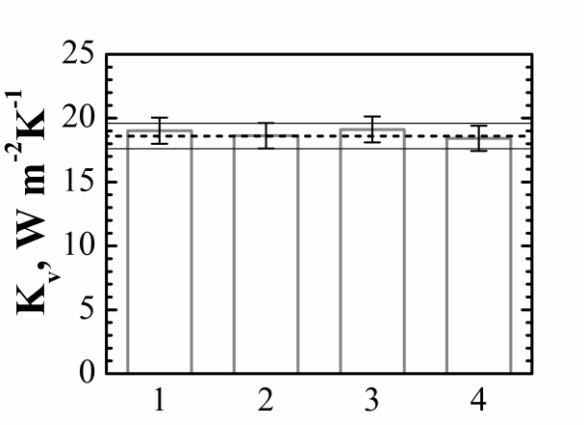


Figure 3

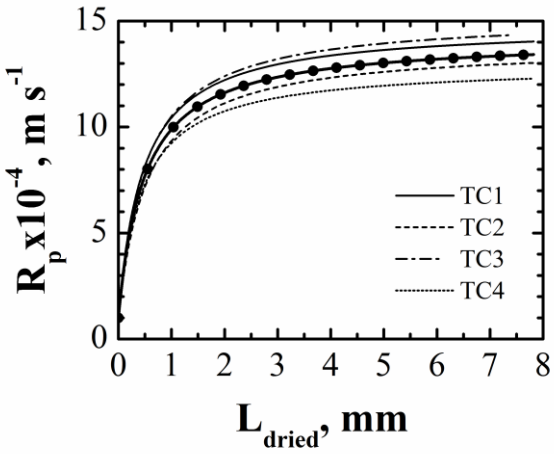


Figure 4

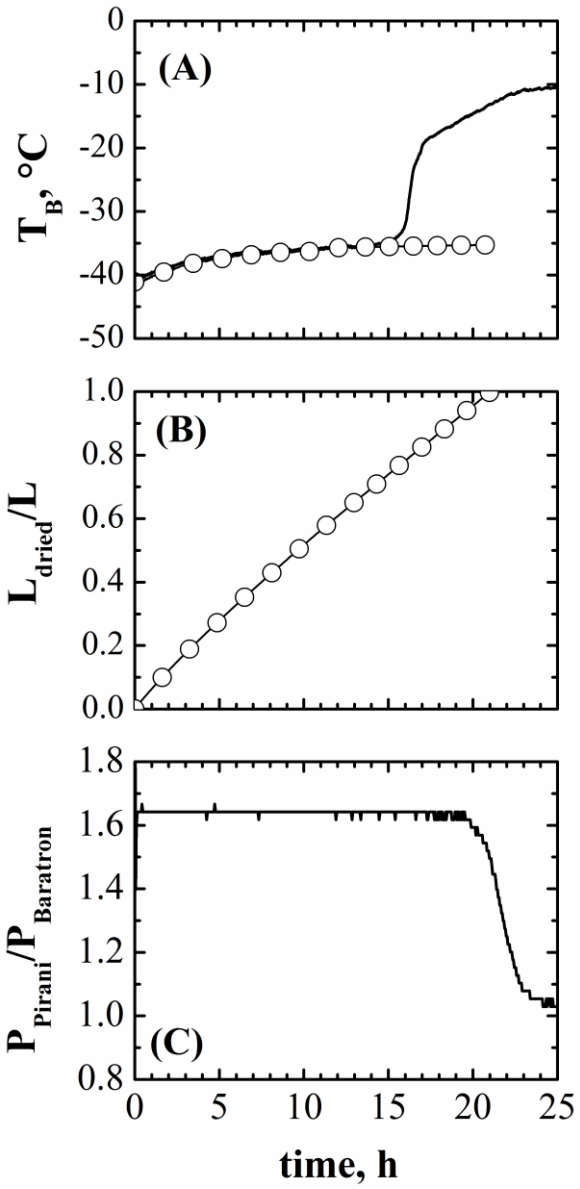

(A)
(B)
(C)
T_B, °C
L_{dried}/L
$P_{Pirani}/P_{Baratron}$
time, h
0
-10
-20
-30
-40
-50
1.0
0.8
0.6
0.4
0.2
0.0
1.8
1.6
1.4
1.2
1.0
0.8
0
5
10
15
20
25

Figure 5

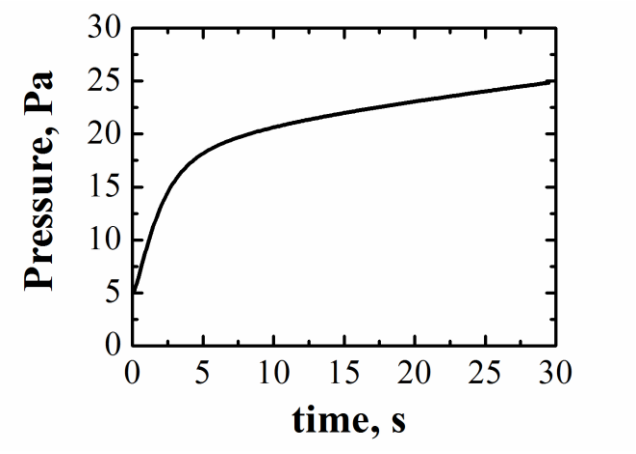


Figure 6

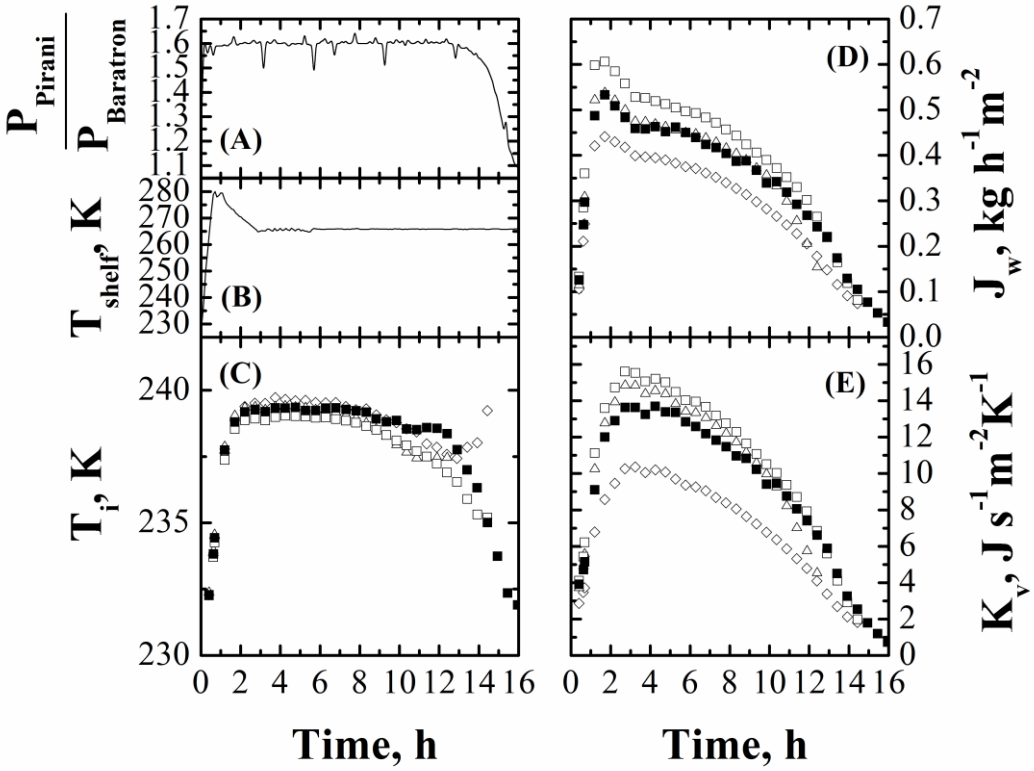

P_Pirani / P_Baratron
(A)
T_shelf, K
(B)
T_i, K
(C)
(D)
J_w, kg h^-1 m^-2
(E)
K_v, J s^-1 m^-2 K^-1
Time, h
Time, h

Figure 7

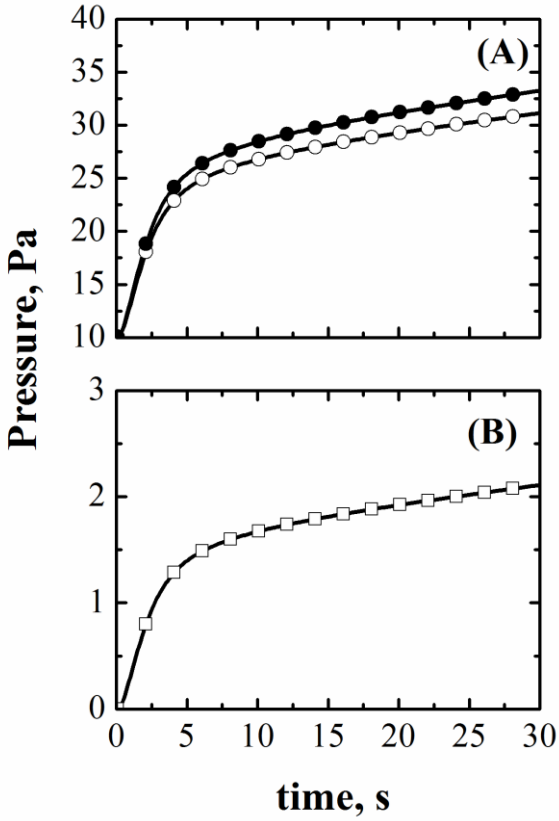


Figure 8

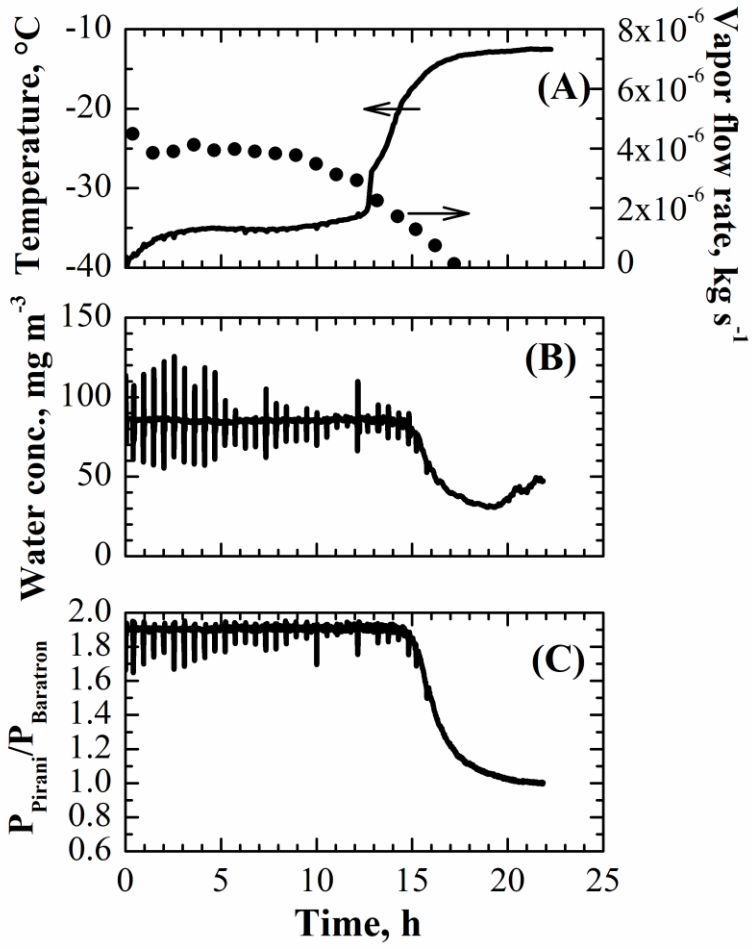
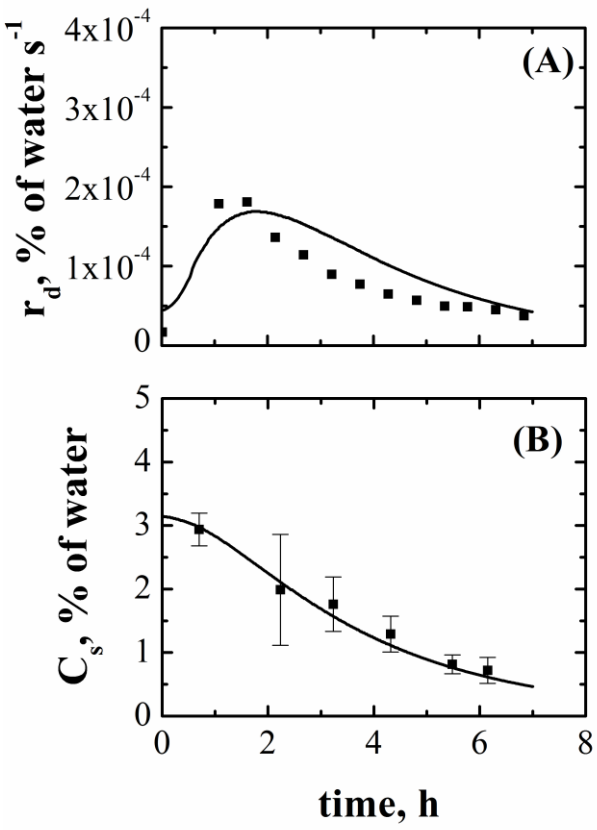

Temperature, °C
Vapor flow rate, kg s-1
(A)
(B)
(C)
Water conc., mg m-3
PPirani/PBaratron
Time, h
-10
-20
-30
-40
8x10-6
6x10-6
4x10-6
2x10-6
0
150
100
50
0
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0
5
10
15
20
25

Figure 9


(A)
(B)
r_d, % of water s^{-1}
C_s, % of water
time, h
4x10^{-4}
3x10^{-4}
2x10^{-4}
1x10^{-4}
0
5
4
3
2
1
0
0
2
4
6
8